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GENERAL PUBLIC EXPERTISE NOTE ON VACCINES USING GMO TECHNOLOGIES

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I. Reminders of some concepts and terminology

1. The cell, DNA, RNA, proteins

DNA is the carrier of genetic information. It is located in a particular compartment of each cell of the body, the nucleus, which is separated from the rest of the cell (the cytoplasm) by a biological membrane, called the nuclear membrane and containing openings (nuclear pores). Each cell is itself delimited by a biological membrane called the plasma membrane and made up of a bilayer of lipids (Figure 1). Genetic information is the same in all cells in the body of a single individual and is distributed over several entities called chromosomes. In humans, the number of different chromosomes is 23. They are present in duplicate in each cell (with the exception of spermatozoa and ova where they are only in single copy), i.e. 46 chromosomes (23 pairs each comprising one chromosome from the father and the other from the mother). All of the chromosomes in a living organism make up its genome. Each chromosome consists of two parallel strands surrounded around an axis to form a double helix: the DNA double helix. Each of the two strands of this double helix is itself made up of four smaller molecules, the nucleotides, which are designated by their respective initials, that is, four letters: A, G, C, T (Figure 1).

Genes are segments of chromosomes (pieces of four-letter sequences) that hold biological information (s) that allow cells to function. The same gene in the same species can exist in different forms, with slight changes in sequence: these are the different alleles of a gene. The alleles of all of an individual's genes determine his genotype. For most of the genes we know today (and which are only a very small part of a genome), the genes hold the secret to making proteins. Proteins are large molecules made up of, not four, but 20 different molecules: the amino acids (Figure 1). The transition from a gene to a protein therefore corresponds the shift from a four-letter language (A, G, C, T: genetic language) to a 20-letter language (the 20 amino acids: protein language). This process is called translation.

However, the passage from gene to protein is not direct: it requires an intermediate molecule, also made up of a sequence of four nucleotides (genetic language A, G, C, U instead of A, G, C, T) and formed from a single strand (single helix). It is an RNA molecule, also called a transcript because it is the result of a process called transcription (passage from DNA to RNA), that takes place in the nucleus. This RNA molecule will then leave the nucleus, through the nuclear pores, to reach the cytoplasm where it will be translated into protein. The expression of a gene encoding a protein therefore takes place in two steps, the first (nuclear) being transcription and the second (cytoplasmic) translation (Figure 1).

Note that not all genes code for proteins. In other words, gene expression does not always include a translation step, but systematically goes through a transcription step. The transcripts are therefore not always intermediates, but can be the end-products of gene expression.

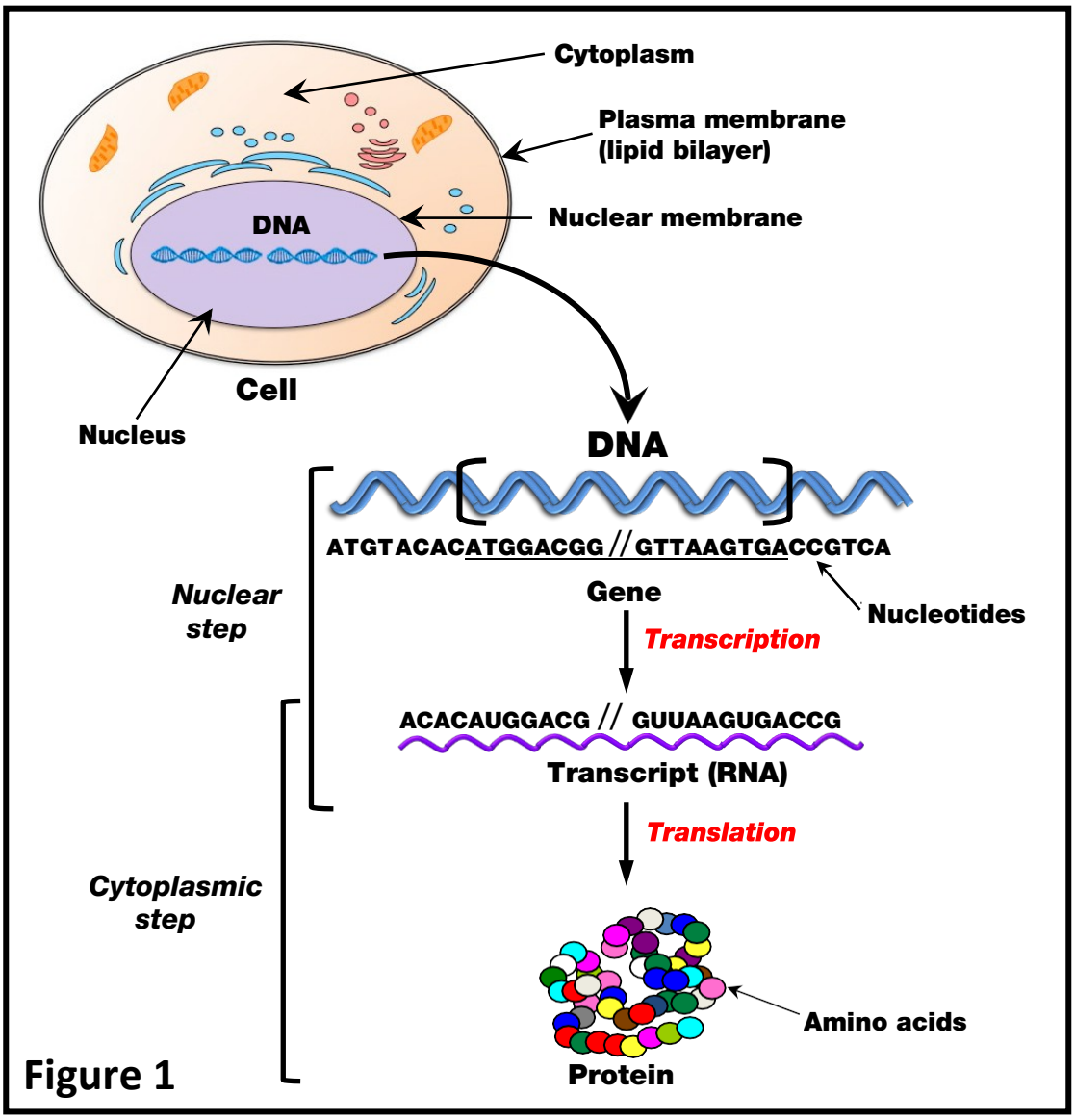

Figure 1

2. Viruses

Viruses are infectious agents made up of a protein shell called a capsid, made up of the juxtaposition of a large number of copies of a viral protein. This capsid contains the genetic material of the virus (Figure 2) which is either DNA or RNA. Many viruses are also surrounded by an envelope which consists of a lipid bilayer corresponding to that of the plasma membrane of their host cells (cells that they infect) and containing embedded proteins: the surface proteins of the virus (Figure 2). Viruses with an envelope are called enveloped viruses; those do not have such an enveloppe are the naked viruses (Figure 2). The SARS-CoV-2 virus, responsible for Covid-19, is an enveloped virus, as is the influenza virus or HIV, responsible for AIDS.

Viruses do not have the capacity to reproduce on their own and must necessarily infect host cells whose activity they divert for the benefit of their own multiplication. For this, the viruses inject their genetic material into the cells they infect. These cells will then replicate this genetic material and express the viral genes it contains in order to produce viral proteins in large numbers. Many viral particles will then reconstitute themselves inside the infected cell.

For naked viruses, the recognition of the host cells is done through the proteins of the capsid which will interact specifically with one or more protein(s) located in the plasma membrane of the host cells. This interaction allows the virus to anchor to the surface of the plasma membrane and then penetrate into the infected cells. In the case of enveloped viruses, recognition and anchoring is by the surface protein, and penetration of the virus, by fusion between the viral envelope and the plasma membrane of the host cell.

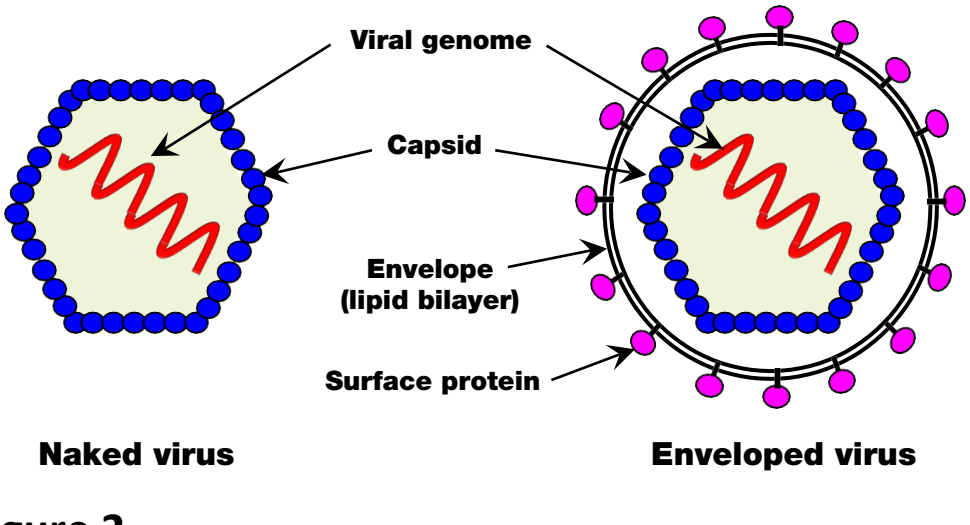

Figure 2

Once inside the infected cell, the viral genetic material will be managed there, according to different mechanisms, depending in particular on its nature: DNA or RNA.

For DNA viruses, viral DNA is taken directly into the machinery of the infected cell, in order to replicate it, and express its genes to produce viral proteins.

In a number of cases, viral DNA can also integrate itself in the genome of infected cells (such as the papillomavirus which causes uterine cancer).

Regarding RNA viruses, there are two main cases.

For some RNA viruses, such as HIV, viral RNA is first transformed into DNA by the action of a viral enzyme, reverse transcriptase, which is injected into the infected cell with viral RNA. This step is therefore the reverse of a transcription, which consists of making RNA from DNA (Figure 1). The resulting viral DNA enters the nucleus through the nuclear pores and then integrates itself in the genome of infected cells. For a great efficiency, this second step again requires a viral enzyme: the integrase. The infected cell can then take over the viral DNA as if it were its own and transcribe it in a large number of copies, some of the transcripts will be translated in order to produce viral proteins.

For other RNA viruses, such as SARS-CoV-2, viral RNA is directly managed by the cellular machinery to translate it and thus manufacture the various viral proteins, including the enzyme required for the replication of this RNA in a large number of copies. In this case, therefore, there is no presence or production of viral DNA, and therefore no integration of the viral genome into the infected cells. This is also the case with the influenza virus, even though its viral RNA cannot be translated directly by the cellular machinery, and must first be replicated as a complementary copy by a viral enzyme released directly into the cell with the RNA.

II. Vaccination

The aim of the vaccination is to stimulate the immune defence of a human or an animal, vis-à-vis an infectious agent, by voluntarily exposing it to this agent (in an attenuated or inactivated form) or to one of its components, called an antigen (usually a protein).

For viruses, most vaccines so far consist of injecting an attenuated ("live vaccines") or inactivated ("inactivated vaccines") form of the whole virus.

The attenuation is obtained mainly by two methods. The first is to cultivate the virus on to cultures of cells of another species: it then remains immunogenic, but can no longer multiply in humans. This is the process used in particular for vaccines against measles, mumps, rubella, chicken pox. The second method consists in using either heat-sensitive mutants of the virus, or viruses adapted to cold, after successive passages in cell cultures at low temperature: these viruses then have a very reduced capacity to multiply at 37 ° C (and therefore in human). This process was used in particular for an old vaccine against the influenza virus, administered by the nasal route (Fluenz®, today withdrawn) and for respiratory syncytial virus (RSV) vaccine. The main drawbacks are on the one hand the risks of the appearance of virus revertants (wild strain) by recombination between the vaccine strain and a pathogenic strain present in the host vaccinated (i.e. a reacquisition of pathogenicity by the vaccine strain initially attenuated), and on the other hand, a contraindication in immunocompromised people or in pregnant women, due to a risk of insufficient attenuation for these people.

The use of inactivated viruses is therefore safer (but not without risk, however: see IV.1). Inactivation is either chemical (mainly a formaldehyde treatment) or physical (heat or irradiation). This type of so-called "inactivated" vaccines concerns in particular influenza, hepatitis A, polio, rabies. Their disadvantage is that they elicit a weaker immune response, which requires multiple and repeated injections, as well as the use of adjuvants, such as added aluminium, to potentiate the immunogenic effect of the vaccine. Such adjuvants may cause toxic effects.

Since the 90s, some vaccines have been obtained using biotechnology. Currently, this consists of using laboratory cultured cells (mainly cells of bacteria, yeasts or filamentous fungi) to make them produce a protein of an infectious agent (antigen). These cultured cells are therefore transgenic cells in the genome of which has been inserted the gene of the infectious agent encoding this antigen. The antigen in question is then purified and combined with various adjuvants to develop a vaccine that will be injected into patients. This is particularly the case with the Engerix ™ -B vaccine against hepatitis B, where the surface protein of this virus was produced in cells of a transgenic yeast (baker's yeast), expressing the viral gene in question. The cost of production is relatively high, due in particular to the step of purification of the antigen, from the transgenic cells that produce it.

Finally, new strategies also involving biotechnology have been under development for several years. They involve causing the antigen of the infectious agent, to be produced directly by the cells of the host, (of the person being vaccinated), by injecting him with the DNA or RNA encoding the viral protein in question.

Getting this genetic material into the host's cells requires the use of "vehicles" called vectors. These vectors are either plasmids-based molecules, or lipid nanoparticles, or genetically modified (GM) viruses. Plasmids are bacterial small circular DNA molecules into which the viral DNA encoding the antigenic protein has been introduced. Lipid nanoparticles

are extra-small lipid bilayers in which the RNA encoding this antigen is trapped. or 3) GM viruses are "disarmed" viruses, that is to say, viruses rendered in particular incapable of replicating, by elimination of part of their genetic material (DNA or RNA), which is replaced by the genetic material of interest which has to be introduced into the host's cells. In the latter case, we then use the natural ability of the viruses in question to inject the genetic material they contain, into human cells.

III. Covid-19 vaccine projects

According to the World Health Organization (WHO) list, updated to September 22, 2020 [1], 38 vaccine candidates are undergoing clinical trials (phase I, II or III).

a) In the case where the vaccine consists of injecting a virus protein, this protein is produced in laboratory by transgenic cells, into which a genetic construct containing the corresponding viral gene has been introduced. The genetic construct allows the production of the viral protein in large amounts in the transgenic cells which are grown on a large scale in fermenters (bioreactors). The protein is extracted from these cells and purified. This concerns 13 of the 38 vaccines, currently being tested.
b) A virus-like particle (VLP) is in fact the capsid without the viral genome, obtained by the spontaneous assembly of the capsid protein, which is produced in transgenic cell grown in a large scale in laboratory. In this specific case (only 1 of the 38 tested vaccines), it concerns plant cells.
c) The remaining 18 vaccines therefore consist of introducing viral genetic material into the cells of the person to be vaccinated (administration is essentially intramuscular, or even intradermal in two of the cases). It is either RNA trapped in lipid nanoparticles (6 cases), either DNA inserted into a plasmid (4 cases), or DNA or RNA delivered by a disarmed genetically modified virus (8 cases).

IV. Analysis of the risks associated with each type of candidate vaccine against Covid-19

1. Inactivated vaccines

The fact that a vaccine uses an inactivated virus, does not mean that there is no risk. The immunizing effect of this type of vaccine is less than with an attenuated virus. It therefore requires repeated injections and the addition of adjuvants, potentially exhibiting toxic effects, to potentiate the immunogenic effect (see part II). A 2004 Swiss study [2] showed that an inactivated influenza vaccine, administered intranasally, caused Bell's palsy (paralysis of all the muscles of the face) in a large number of patients, without however we know the exact reason. This vaccine has since been withdrawn.

In addition, several studies have shown an increased risk of infection (with the same virus or others) following vaccination with inactivated vaccines. This is the case with the influenza vaccines, Vaxigrip and Fluzone. For the first, in 2012 it was shown to increase the risk of infection with other respiratory viruses in children aged 6 to 15 [3]. The second has been shown to increase the risk of influenza infection in obese adults (compared to non-obese people themselves vaccinated) [4]. More recently, in 2019, a study found that an inactivated dengue vaccine increased the rate of infection with the same virus in macaques [5]. This

phenomenon had previously been observed with an attenuated virus, in children not exposed to dengue fever before vaccination [6].

Special precautions must therefore be taken with inactivated vaccines against Covid-19, especially since the virus responsible for it is completely new, and we are far from understanding all the effects.

2. Vaccines containing the antigenic protein and VLP vaccines

In addition to the cost that they represent, due to the more or less cumbersome stage of purification of the viral protein from the transgenic cells which produce it, these vaccines prove to be ineffective and may exhibit toxic effects. Those effects are mainly due to the adjuvants (such as aluminium or formaldehyde for example), added precisely to compensate low efficiency and therefore potentiate the stimulation of the immune system, but also possibly to the antigen itself. The antigenic protein being produced by transgenic cells (which are therefore not those which produce it normally), may present structural or chemical differences which may give it unexpected properties. Indeed, if the genetic message contained in the viral gene (transgene) dictates to the cells that host it (transgenic cells), during the translation process, the nature and sequence of amino acids to make the viral protein (antigen), it is however only very partially responsible for how the protein must fold in space. This folding depends in part on the nature and sequence of amino acids (and therefore of the gene), but mainly on the environment of the cell, in which the protein is made (acidity, salt concentration, etc.). However, the cellular environment can vary considerably from one cell type to another and we can never be sure that the protein of interest (here the viral antigen) is correctly folded, when it is artificially produced by the transgenic cells-- even when this protein retains the biological activity of interest (here, its immunogenic character) [7]. Wrong folding of a protein can have absolutely unpredictable and sometimes very unfortunate consequences. Let's not forget that prion diseases, for example (mad cow disease, Creutzfeldt-Jakob disease, scrapie, etc.), are due to simple folding defects of a particular protein. Certainly, not all folding defects make prions ..., but let us pray that the viral protein folds well.

Moreover, once its folding is complete, the protein can be the subject of secondary chemical modifications (called "post-translational") such as additions of sugars, phosphates, which may be necessary for its functionality, its activity, or him confer particular properties such as - precisely - immunogenic properties. Here again, we will never be sure that these post-translational modifications (which are not "dictated" by the gene) put in place in the transgenic cells, are absolutely identical to what they are in the cells which naturally produce the protein (here, cells naturally infected by the virus) [7].

3. Vaccines delivering the RNA or DNA encoding the antigenic protein.

3.1. The risk of the appearance of recombinant viruses

This risk is independent of the vector used to deliver the viral DNA or RNA encoding the protein antigen, into the host cells, whether it is a plasmid vector, a nanoparticle or a genetically modified virus. However, this risk is even greater in the case of the use of genetically modified viruses, because they provide not only the viral DNA or RNA of interest, but also part of their own genome.

Viruses have a great capacity to exchange fragments of their respective genetic material, as long as the viral genomes concerned are of the same nature (either DNA or RNA), and that they share similar sequences (genes). The well-known process that governs these exchanges is called recombination (and when this recombination takes place between DNA or RNA sequences that resemble each other, this is called homologous recombination). This recombination phenomenon is not reserved for DNA or viral RNA, but the viral sequences are known to undergo numerous recombinations (they are said to be very "recombinogenic"). It results from these recombinations — between viral genetic materials — so-called "recombinant" viruses —, of which the genes which have been the site of these exchanges are called "mosaics". This means that they are made up of partly sequences from virus 1 and sequences from virus 2 (Figure 3). Figure 3 illustrates the recombination between DNA viral, but this phenomenon can occur just as well between viral RNAs.

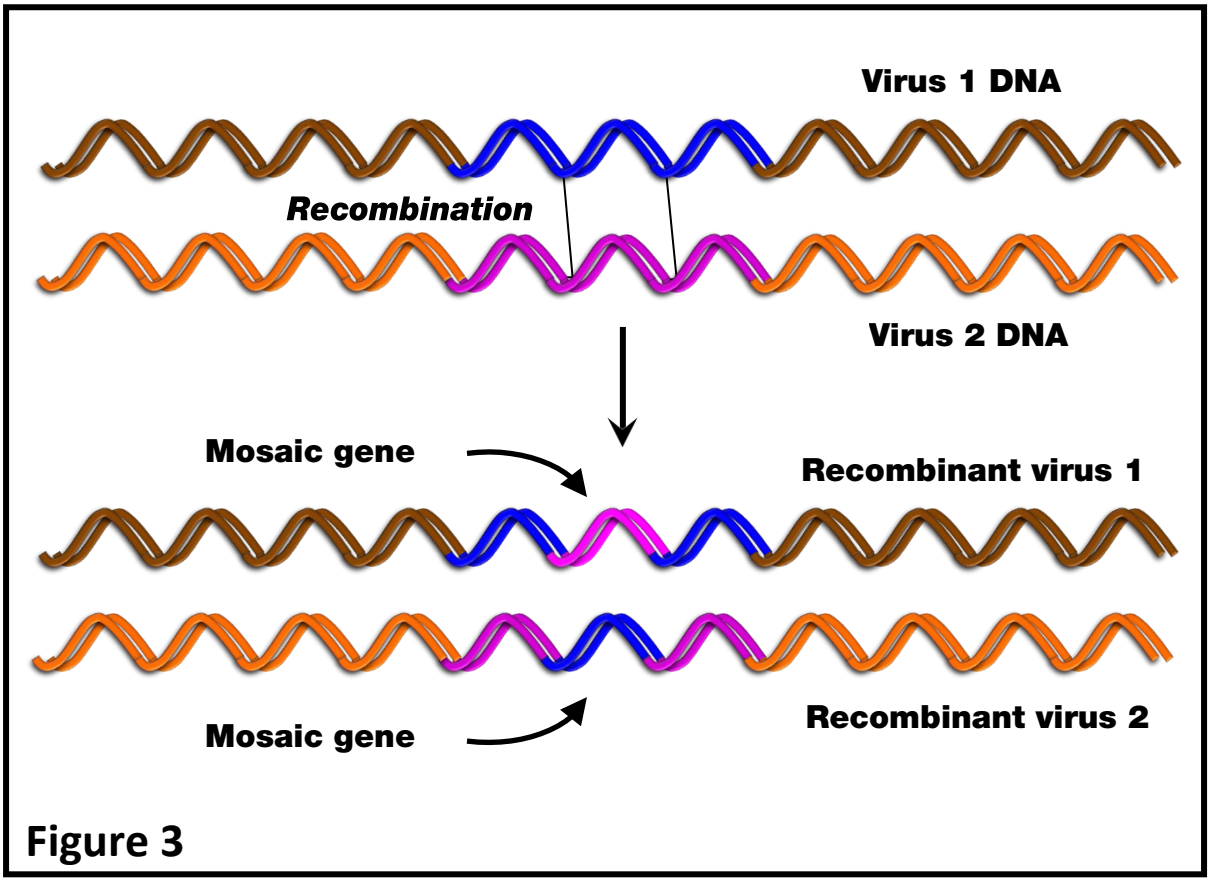

Figure 3

In a number of cases, these recombinant viruses are much more virulent than the original viruses and can therefore cause aggravated viral infections. This phenomenon was widely demonstrated in transgenic plants — in the genome of which a viral gene has been voluntarily

introduced — infected with a virus related to that from which the viral transgene originates [8-16]. A high profile example of a recombinant virus that can cause aggravated viral infections in humans is that of the 2009 H1N1 virus, recombinant between three strains of influenza virus: a pig strain, a human strain and an avian strain [17, 18].

Of course, this phenomenon can only occur if genetic material from at least two viruses is found in the same cells, which is fortunately extremely rare in nature since it implies that the same cells are co-infected with at least two viruses. But under the influence of man, this phenomenon can become much more common. This is of course the case, as mentioned above, with transgenic plants into which a viral transgene has been introduced, where it is sufficient that these plants are infected with a single virus for such recombination events to occur. But it is also the risk that we run in humans, when we generate vaccines delivering viral RNA or DNA, into patients' cells. Covid-19 candidate vaccines of this type — actually tested in clinical trials — are administered intramuscularly or intradermally. The target cells are therefore muscle cells, skin cells, fibroblasts (connective tissue cells, that is to say the supporting tissue that envelops organs, tissues, and in particular muscle bundles), but also circulating blood cells and endothelial cells (which border blood vessels). These cells can also be targets of infection from other viruses. For example enteroviruses (naked RNA viruses) have been detected in muscle cells [19], Zika virus infects skin cells [20], Chikungunya targets satellite muscle cells (stem cells for muscle tissue) [21], but also endothelial cells and fibroblasts [22]. And these are probably just a few examples…

Vaccination against Covid-19, if it becomes a reality, will be mass vaccination across the world. The probability that this kind of event will occur is therefore far from being zero, even if it remains undoubtedly low in terms of frequency. Such mass vaccination with this type of vaccine could become a large-scale factory for new viruses recombinants. Let's not forget that it is enough for a new virus to appear somewhere in the world for the health, environmental and social consequences to be global and colossal.

3.2. The risk of insertional mutagenesis (genotoxicity)

Insertional mutagenesis is a mutation (modification of genetic information) by insertion of a sequence, inside a genome. This insertion can then inactivate or modify the expression of one or more gene (s).

This risk of genotoxicity for the human cells targeted for vaccination (whose genome is of course DNA), therefore only concerns vaccines delivering viral DNA, whether the vector is a plasmid or a genetically modified virus. However, this risk may also concern vaccines delivering RNA, by means of a genetically modified RNA viral vector of the type of the AIDS virus (HIV, widely used as a vector), if this has not been correctly deprived of its reverse transcriptase and its gene encoding it. The viral reverse transcriptase can then convert the delivered RNA into DNA, which will integrate into the genome of the target cells.

Genetically modified viruses are also widely used for gene therapy purposes. In this case, they deliver the normal version of a human gene that turns out to be defective (mutated) in the patient being treated. In 2002, three years after a gene therapy trial (in children with severe immunodeficiency, due to a mutation in a gene on the X chromosome) using a genetically modified RNA virus as a vector, two of the 10 children treated developed leukemia, due to the insertion of repair DNA — delivered by the viral vector — in proximity to a proto-oncogene (cancer gene), causing a severe disruption of its expression [23]. Several studies have shown the effects of insertional mutagenesis caused by different families of RNA viruses (including HIV) [24]. Likewise, several studies carried out in mice have shown that the delivery of genes by vectors derived from the adeno-associated virus (AAV, a small non-pathogenic

DNA virus) results in insertional mutagenesis [25]. In 2016, a study on the genotoxic effects of viral vectors, derived of HIV and AAV, used for gene therapy purposes, concluded that "an in-depth knowledge of viral biology and advances in cell genetics are needed to elucidate the nature of the selection of sites for integration of viral vectors and the risks associated to it" [26].

4. Risks specifically related to the use of modified viral vectors: immunotoxicity

In addition to the risks of the appearance of recombinant viruses and insertional mutagenesis (especially when the genetic material delivered is DNA), the viral vectors are themselves immunogenic. They can cause significant effects of immunotoxicity.

In 2002, a pilot gene therapy experiment, carried out in 18 boys suffering from a severe metabolic disorder, due to a defective gene located on the X chromosome, led to the death of an 18-year-old young man. This death was due to a fatal systemic inflammatory disease, caused by the viral vector (disarmed human DNA virus): DNA sequences of the vector have been found in most of its tissues [27]. The fact that the 17 other individuals, treated absolutely did not show this type of response, shows how difficult it is to predict, and therefore control this risk. In Belgium, several clinical trials of immunotherapy to fight cancer and using a disarmed virus, where more than 15% of its genome has been replaced by two human genes (encoding an antigen present on the surface of cancer cells and an interleukin, a protein communication between immune cells), showed non-specific activation of the immune system, linked to the vector. This resulted into an inflammatory reaction and an autoimmune response [28]. Numerous other studies have shown immunotoxicity effects of various viral vectors used for the purpose of gene therapy or vaccination [29-33]. In the case of viral vectors used for vaccination purposes, anti-vector immunity can also directly interfere with the desired vaccine efficacy (vaccine immunogenicity) [34].

V. General considerations relating to the risk assessment of these vaccines

The use of vaccines delivering viral genetic material (DNA or RNA) is new or recent. The use of genetically modified viruses as vectors, in particular for the purposes of gene therapy or immunotherapy, has shown the extent to which the adverse effects are varied, not mastered and can be serious. If the attempts at immunotherapy are relatively recent, the failures of gene therapy since nearly 35 years are here for us to remind. These failures can be explained in large part by the search for the scoop, at the expense of efficacy and/or biosafety. Such an approach will never meet the expectation and the needs in terms of care.

But, the use of these same vectors for vaccination is yet another dimension. Indeed, gene therapy or immunotherapy affects not only a limited number of people, but also seriously ill people. Therefore, not only the possible side effects concern a small number of individuals, but the seriousness of their state of health and the health emergency, in which they find themselves, no doubt allows them to accept a certain risk-taking. In the case of vaccines, we are in a preventive approach. This therefore concerns a considerable number of people, the vast majority of whom are in good health (in any case with regard to the pathology of which the vaccine is supposed to protect us). Uncontrolled side effects would therefore have considerable consequences, especially in a mass vaccination campaign, such as the one intended to fight Covid-19. These repercussions could be disastrous on the health level of course. But, also on the environmental level (in the case for example of the propagation of

new recombinant viruses: see section IV.3.1) And, the fact that it is a prevention approach, does not allow any risk taking.

Therefore, these vaccine candidates require a thorough health and environmental assessment, incompatible with the urgency, whatever the origin of this urgency: pressure from decision-making and health authorities, or profits of the pharmaceuticals industry competing for the vaccine. In its framing note of July 23, 2020 on the vaccine strategy against Covid-19 [35], the High Authority of Health (HAS) declares: "In the context of the Covid-19 pandemic, the stake is therefore to design the most effective and safest vaccine possible in record time". This claim is nonsense and an aberration on the part of an authority, such as HAS.

The dangers associated with the characteristics of genetically modified viral vectors, or their possible dispersion or dissemination, must be addressed in the context of an assessment, extremely restrictive environmental risk.

On the contrary, articles 2 and 3 of the very recent European regulation 2020/1043 state that any clinical trial of medicinal products containing GMOs or consisting of such organisms, and intended to treat or prevent Covid-19, can escape, preliminary assessments on health and the environment. This opens the door to the greatest laxity in terms of assessment and goes completely against the precautionary principle.

In addition, this regulation calls into question, in fact, the containment legislation that applies to genetically modified microorganisms and viruses. This regulation defines 4 levels of containment (identified from 1 to 4: the higher the number, the higher restrictive containment). Handling pathogenic viruses requires a minimum confinement of 2, very often 3, or even 4. The provisions of regulation 2020/1043 open the door to containment zero, even before demonstrating the health and environmental safety of the genetically modified viruses in question.

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